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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
09/687,246	10/12 2000	William G. Nelson	JHU1660-1	1350

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EXAMINER	
SOUAYA, JEHANNE E	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 04/23/2002

#17

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
09/687,246	Nelson et al
Examiner	**Art Unit**
Jehanne Souaya	1634

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on *Nov 21, 2001* .

2a) ☐ This action is **FINAL.** 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *1-51 and 76-85* is/are pending in the application.

4a) Of the above, claim(s) *76-83* is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *1-51, 84, and 85* is/are rejected.

7) ☒ Claim(s) *8, 84, and 85* is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)

16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

17) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). *10, 12*

18) ☐ Interview Summary (PTO-413) Paper No(s). _____

19) ☐ Notice of Informal Patent Application (PTO-152)

20) ☐ Other:

DETAILED ACTION

Please note that the art unit designation for the examiner has changed from 1655 to 1634.

Election/Restriction

1. Applicant's election with traverse of Group I, claims 1-51, 84 and 85 in Paper No. 16 is acknowledged. The requirement is still deemed proper and is therefore made FINAL.

2. Claims 76-83 have been withdrawn from consideration as being drawn to a non-elected invention. An action on the merits of claims 1-51 and 84-85 follows.

Priority

3. Applicant's claim for priority under 35 USC 119(e) to provisional application 60/159,168, filed October 13, 1999 is acknowledged. The claims have been awarded an effective filing date of October 13, 1999 as the subject matter in the claims is taught in the '168 application.

Drawings

4. The drawings have been approved.

Claim Objections

5. Claim 8 is objected to because of the following informalities: the term "oligonucleotide" is misspelled as "oligonucleitide".

Claims 84 and 85 are objected to because they are dependent on claim 52, which has been canceled.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

Indefinite

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claim 1-51, 84, and 85 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claim 1 is indefinite in the recitation of "hepatic cell proliferative disorder" as it is unclear if the term encompasses only hepatic cell cancer, or any cell proliferative disorder of the liver, such as cysts. The specification does not define the term, therefore, the metes and bounds of the claim are unclear.

B) Claims 1, 2, and 40 are indefinite in the recitation of "glutathione-S-transferase nucleic acid" or "GST nucleic acid" as it is unclear if the term encompasses only nucleic acid encoding glutathione-S-transferase, or to a chimera comprising GST polypeptide attached to a nucleic acid. The specification does not define the term, therefore, the metes and bounds of the claim are unclear.

C) Claim 4 is indefinite in the recitation of "the start site" as the term lacks sufficient antecedent basis. Further, the claim does not make clear as to what start site is encompassed by the claim.

D) Claim 6 is indefinite in the recitation of "the methylated nucleic acid" in line 7, as the term lacks sufficient antecedent basis. It is unclear if the term refers to 'methylated CpG containing glutathione -S-transferase nucleic acid" from claim 1 or if it refers to the modified methylated cytosine of claim 6, line 6.

E) Claims 6 and 7 are indefinite in the recitation of "said amplifying step" and "the amplifying step" as there is insufficient antecedent basis for the recitation of "amplifying step".

F) Claim 8 is indefinite in the recitation of "have a sequence as set forth *in* SEQ ID NO:..." as it is unclear if the term encompasses sequences consisting of the recited SEQ ID NOS or sequences *within* the recited SEQ ID NOS. The specification does not define the term, therefore, the metes and bounds of the claim are unclear.

G) Claim 11 is indefinite in the recitation of " the CpG containing nucleic acid" as there is insufficient antecedent basis for the term "CpG containing nucleic acid".

H) Claim 14 is indefinite as it is unclear where in the method steps of claim 6, the method step of claim 14 is carried out.

I) Claim 16 is indefinite in the recitation of "the GST" as the term "GST" lacks sufficient antecedent basis. It is unclear if the term refers to "GST nucleic acid" or just "GST". It is noted that this recitation occurs in claims 18, 20, 27, 51. There is insufficient antecedent basis for the term "GST target nucleic acid" as well.

J) Claims 16 and 28 are indefinite in the recitation of "GST DNA" and GST RNA". It is unclear if the term encompasses only nucleic acid encoding glutathione-S-transferase, or to a

chimera comprising GST polypeptide attached to a nucleic acid. The specification does not define the term, therefore, the metes and bounds of the claim are unclear.

K) Claims 27, 39, and 51 are indefinite as it is unclear if the claim intends that the methylation status of GST is compared to the methylation status of GST in adjacent normal hepatic tissue, or to the methylation status of the hepatic tissue itself.

L) Claims 41 and 42 are indefinite in the recitation of "the nucleic acid" as the term lacks sufficient antecedent basis. It is unclear if the term refers to "GST nucleic acid".

Enablement

8. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. Claims 1-51, 84 and 85 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of detecting hepatocellular carcinoma (HCC) or liver cancer in humans comprising obtaining a specimen that contains a nucleic acid encoding GSTP1, wherein the specimen is selected from the group consisting of hepatic tissue, bile, and blood, and detecting a hypermethylated CpG promoter region at -539 to -239 from the transcription start site of the nucleic acid encoding GSTP1, wherein a hypermethylated CpG promoter region at -539 to -239 from the transcription start site of the nucleic acid encoding GSTP1 in the specimen as compared to the level of methylation of the promoter region at -529 to

-239 from the transcription start site of the nucleic acid encoding GSTP1 in normal hepatic tissue, is indicative of hepatocellular carcinoma or liver cancer in humans; does not reasonably provide enablement for a method for detecting a hepatic cell proliferative disorder comprising detecting a methylated CpG containing GST in a biological fluid or detecting a decrease in the level of GST RNA as compared to GST RNA levels in normal cells. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

There are many factors to be considered when determining whether there is sufficient evidence to support determination that a disclosure does not satisfy the enablement requirements and whether any necessary experimentation is undue (See *In re Wands,* 858 F. 2d 731, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). These factors include, but are not limited to:

Quality of Experimentation Necessary
Amount of Direction and Guidance
Presence and Absence of Working Examples
Nature of the Invention
Level of predictability and unpredictability in the art

The claims are broadly drawn to detecting any hepatic cellular proliferative disorder in any organism by detecting methylation of any region of any GST nucleic acid or by detecting decreased levels of any GST RNA in any biological fluid. The claims are further limited to detecting hypermethylation of GST DNA in any region as well as a promoter, and more specifically to the region -539 to -239 from the start site (presumably the transcription start site). The claims are also further limited to a π family GST and more specifically to GSTP1. The

claims are also limited to detecting methylation of any GST nucleic acid and hepatitis B or C virus and correlating such to any hepatic cellular proliferative disorder. The specification, however, does not enable the skilled artisan to make or use the invention commensurate in scope with the claims. Although some claims are of a more limited scope, they are still too broad such that the specification does not enable the broad scope of the claims.

The specification teaches that the invention is based on the observation that human liver carcinogenesis proceeds via accumulation of CpG island hypermethylation changes at GSTP1 and that such hypermethylation was detected in 85% of HCC cases studied. The specification (p. 36) teaches that Hep3B HCC cells tested, failed to express either GSTP1 polypeptides or GSTP1 mRNA (para 1). The specification further teaches that GSTP1 promoter alleles present in Hep3B HCC cells manifested abnormal hypermethylation. The specification teaches that when Hep3B HCC cells were exposed to 5-aza-dC, an inhibitor of DNA methyltransferases, GSTP1 mRNA expression increased (para 2). The specification, at page 38, teaches that 19 of 20 HCC cases appeared devoid of GSTP1 polypeptide expression (table 1) and that DNA from 17 of the 20 HCC specimens showed somatic hypermethylation changes in at least 1 GSTP1 allele (p. 40. Ines 12-13). The specification further teaches that 10 of 20 HCC cases had detectable hepatitis B virus DNA (HBV) among genomic DNA from HCC tissue or from adjacent tissue (p. 41) and that abnormal GSTP1 promoter DNA hypermethylation was present in HCC DNA in 7 out of 10 cases in which HBV was present and in 10 out of 10 cases in which HBV DNA was not detected.

The claims are broadly drawn to detecting any hepatic cell proliferative disorder, however the only proliferative disorder analyzed by the specification is hepatocellular carcinoma. The specification provides no guidance or working examples that any hepatic cell proliferative disorder, such as a cyst, can be detected by detecting any methylated CpG containing nucleic acid encoding GST, or any hypermethylated CpG containing nucleic acid encoding GSTP1, or a decrease in the level of GST RNA. With regard to detecting a methylated CpG containing GST nucleic acid in any biological fluid, the specification provides no guidance or working examples that hepatocellular carcinoma can be detected by detecting hypermethylated GST nucleic acid or decreased levels of GST mRNA in a urine sample or ejaculate, for example. Given that the specification teaches that the hypermethylation of the GSTP1 promoter region analyzed appeared to be somatic (p. 40, lines 3-4), the specification provides no teaching or guidance that cancerous hepatocytes could be predictably and reproducibly detected in a urine or ejaculate specimen. To practice the invention as broadly as it is claimed, the skilled artisan would have to screen biological fluid samples from a large number of patients with any hepatic cell proliferative disorder, as well as obtaining biological fluid samples from healthy controls to determine whether hypermethylated CpG containing GST or decreased levels of GST mRNA could be detected in any biological fluid sample, and further, if any hepatic cell proliferative disorder could be diagnosed from such samples. Such recitation in the claims provide the skilled artisan with an invitation to experiment. Such experimentation is considered undue, however, as it requires trial and error analysis, the results of which are unpredictable.

With regard to detecting an association between methylated CpG containing GST nucleic acid and any hepatic cell proliferative disorder, the specification provides no guidance or working examples that any hepatic cell proliferative disorder can be detected by only detecting methylated CpG containing GST. Further, Herman et al (US Patent 6,017,704) teaches that human genes are known to be methylated (see col. 4, lines 1-5), including GST pi (human GST pi is GSTP1), and that hypermethylation of GST pi is associated with neoplastic tissue. Therefore, because the art teaches that GSTP1 inherently exists in a methylated state, the skilled artisan would not be able to detect a hepatic cell proliferative disorder based solely on the detection of methylated CpG containing GST nucleic acid. Furthermore, although the specification teaches that DNA hypermethylation was present in HCC DNA in 7 out of 10 cases in which HBV was also detected, the specification does not teach the detection of HCV. Furthermore, the specification does not provide any guidance or working examples that detecting HBV or HCV and detecting methylated CpG containing GST nucleic acid is indicative of a hepatic cell proliferative disorder. Since CpG containing GSTP1 nucleic acid is inherently methylated, the detection of HBV along with methylated GSTP1 could be indicative of hepatitis B infection, but does not necessarily indicate to one of skill in the art that a hepatic cell proliferative disorder, such as liver cancer or a cyst, is also present.

With regard to detecting a hepatic cell proliferative disorder in any subject, the specification provides no working examples that a hepatic cell proliferative disorder in any organism or mammal, such as a rat for example, can be detected by detecting any methylated

CpG containing nucleic acid encoding GST, or any hypermethylated CpG containing nucleic acid encoding GSTP1, or a decrease in the level of GST RNA. The art is unpredictable with regard to such, as although the specification teaches that GSTP1 mRNA was absent in Hep3B cells, Imai et al (Carcinogenesis, vol. 18, pp 545-551, 1997) teach that expression of GST-P mRNA (rat π form of GST) was high in all rat hepatocellular carcinoma samples tested (see abstract, lines 16-18, Fig. 4). Further, Steinmetz et al (Carcinogenesis, vol. 19, pp 1487-1494, 1998) teaches that while eight cytosines between -235 and +140 in the GST-P promoter region were methylated in a site specific manner in GSTP-negative control liver, these same sites were hypomethylated in four chemically induced GSTP-positive neoplasms (see abstract, lines 16-20). Therefore, based on the lack of guidance in the specification as to an association between a hepatic cell proliferative disorder and hypermethylation of CpG in any GST nucleic acid or a decrease in GST mRNA expression in any mammal, for example, the skilled artisan would have to screen a large number of organisms to determine wether a hepatic cell proliferative disorder can be detected in any organism by detecting hypermethylation of CpG in a GST nucleic acid or a decrease in mRNA expression. The result of such analysis is unpredictable given that the art teaches that GST-P mRNA was high in all rat hepatocellular carcinoma samples tested and that while eight cytosines between -235 and +140 in the GST-P promoter region were methylated in a site specific manner in GSTP-negative control liver, these same sites were hypomethylated in four chemically induced GSTP-positive neoplasms. Therefore, the skilled artisan would be required to perform undue experimentation to practice the invention as broadly as it claimed.

With regard to detecting a hepatic cell proliferative disorder by detecting a decreased level of GST RNA in comparison to GST RNA levels in normal cells, although the specification teaches that GSTP1 mRNA was absent in Hep3B cells, the specification also teaches that normal hepatocytes generally fail to express GSTP1. Further, the specification does not teach comparing the level of GST mRNA expression in HCC cases versus normal hepatocytes, therefore, based on the lack of guidance from the specification, and the fact that the specification teaches that normal hepatocytes generally fail to express GSTP1, it is unpredictable as to whether a hepatic cell proliferative disorder, or hepatocellular carcinoma, can be detected by merely detecting a decrease in GSTP1 mRNA expression. The art is further unpredictable with regard to GST expression and hepatocarcinogenesis. De Oliveira et al (Arquivos de Gastroenterolgia, 1990, vol. 27, pp 83-94, English abstract provided) teaches that serum levels of glutathione S transferase was increased in 64% of patients with hepatocellular carcinoma (lines 1-3 of abstract). Therefore, based on the lack of guidance from the specification, and the unpredictability taught in the art with regard to expression of GST in hepatocellular carcinoma, the skilled artisan would have to first determine whether a change in mRNA expression of any GST form (four are taught in the specification, at page 7, line 11: π, μ, α, and θ) exists between hepatocytes from tumor tissue and hepatocytes from normal tissue, to determine if a lack of GSTP1 or [any GST] expression is indicative of a hepatic cell proliferative disorder. Given that the specification teaches that normal hepatocytes generally fail to express GSTP1, such analysis

would require trial and error, the outcome of which is unpredictable, thus constituting undue experimentation.

With regard to detecting methylation or hypermethylation in any region of a GST nucleic acid, the specification does not teach an association between HCC and hypermethylation in any region of the nucleic acid encoding GST, and instead teaches that somatic DNA hypermethylation changes present in HCC DNA consistently affected the gene promoter (p. 7, lines 1-4). The specification also teaches that hypermethylation of the promoter region reduces the expression of GSTP1 in liver tissue (p. 8,line 22). Further, Steinmetz teaches, with regard to rat GST-P, that five of the eight CpG sites between -235 and +140 of the GSTP promoter regions, are located within consensus sequences for the DNA binding proteins Sp1 (whose binding is essential for GSTP transcription) and E2F, indicating at least one possible mechanism that could potentially lead to transcriptional activation of GSTP in hepatocellular foci and neoplasms during rat hepatocarcinogenesis (see abstract, last 3 sentences, and p. 1490). Steinmetz further teaches that methylation of critical cytosines within the promoter region, rather than all CpG associated cytosines, may be a determining factor in regulation of GSTP expression. Therefore, based on the teaching in the specification and the art, regulation of GSTP or GSTP1 can be regulated by hypermethylation of the promoter region. There is no teaching or guidance in the specification, however, that hypermethylation in an intron or exon, for example, of GSTP1 would lead to decreased expression of GSTP1 or be associated with hepatocellular carcinoma. Undue experimentation would be required of the skilled artisan to practice the invention as

broadly as is claimed. The skilled artisan would have to screen a large number of hepatocellular carcinoma samples and normal hepatocytes, and using trial and error analysis, would have determine the level of methylation of CpG dinucleotides in all regions of the gene encoding GSTP1 to determine whether hypermethylation in any region of the gene is associated with HCC or any hepatic cell proliferative disorder. Such analysis would require trial and error, the outcome of which is unpredictable, thus constituting undue experimentation.

Conclusion

10. No claims are allowable.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jehanne Souaya whose telephone number is (703)308-6565. The examiner can normally be reached Monday-Friday from 9:00 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax phone number for this Group is (703) 305-3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Jehanne Souaya

Jehanne Souaya
Patent examiner
Art Unit 1634
April 19, 2002